

Asbestos-Induced Lung Disease

by Arnold R. Brody

This review attempts to deal with two major questions concerning asbestos-induced lung disease: How does inhaled asbestos cause cell proliferation and fibrosis? and Will there continue to be risk from exposure to asbestos in schools and public buildings? The first is a scientific question that has spawned many interesting new experiments over the past 10 years, and there appear to be two hypothetical schemes which could explain, at least in part, the fibroproliferative effects of asbestos fibers. One supports the view that toxic oxygen radicals generated on fiber surfaces and/or intracellularly are the central mediators of disease. The second hypothesis is not mutually exclusive of the first, but, in my opinion, may be integral to it, i.e., the cellular injury induced by oxygen radicals stimulates the elaboration of multiple varieties of growth factors and cytokines that mediate the pathogenesis of asbestosis. There is increasing evidence that molecules such as platelet-derived growth factor and transforming growth factor β , both synthesized and secreted by activated lung macrophages, are responsible, respectively, for the increased interstitial cell populations and extracellular matrix proteins that are the hallmarks of asbestos-induced fibrosis. The challenge today is to establish which combinations of the many factors released actually are playing a role in disease pathogenesis. The issue of continued risk currently is more a question of policy and perception than science because a sufficient database has not yet been established to allow full knowledge of the circumstances under which asbestos in buildings constitutes an ongoing health hazard. The litigious nature of this question does not help its resolution. In as much as public policy statements and risk assessment are not within my purview, I have focused on the state-of-the-art of asbestos as a complete carcinogen. It appears to be generally nongenotoxic, but all asbestos fiber types can induce chromosomal mutations and aneuploidy, perhaps through their ability to disrupt normal chromosome segregation.

Introduction

Asbestos and the diseases it causes have been popular topics over the past 15 years. Numerous books and reviews are available (1-5). The reviews have served scientific and policy issues (6,7), both of which will be discussed here in part.

The term "asbestos" refers to a group of naturally occurring fibrous minerals that have been mined for centuries and used in the manufacture of numerous useful products including construction materials, fireproofing, brake lining, and cement pipes (1,2). The mineral generally is divided into two types, the amphiboles and serpentine varieties. The former group contains four fiber types based on their elemental content and crystalline nature. These are crocidolite, amosite, anthophyllite, and tremolite. Although the serpentine group is populated by only one commercially useful variety, chrysotile, it has been by far the most commonly used fiber type, constituting about 90% of the world's use. The reader is referred to informative texts for further details of asbestos mineralogy and utilization (1,2).

Books and reviews have chronicled decades of primary research designed to establish whether or not asbestos causes lung scarring (fibrosis) and cancer, and more recently the cellular, biochemical, and molecular mechanisms through which it does so. The most current research on asbestos-related disease uses the durable fibers in *in vivo* and *in vitro* model systems, which are defining the basic mechanisms of mineral-fiber-induced lung fibrosis (8,9) and the molecular basis of car-

cinogenesis (10). Numerous questions remain to be answered. In this paper, I try to deal with two questions that remain unresolved today: How does inhaled asbestos cause cell proliferation and fibrosis? and Will there continue to be risk from exposure to asbestos in schools and public buildings.?

How Does Inhaled Asbestos Cause Lung Fibrosis?

Reactive Oxygen Radicals

There are two major hypotheses put forth to explain how asbestos fibers induce a fibrogenic response. One is the oxygen radical hypothesis and the other is the growth factor hypothesis. The former states, in essence, that bioreactive oxygen species and their cytotoxic metabolites are produced extracellularly on fiber surfaces and/or intracellularly as a result of interactions with various lung cells. Support for this hypothesis is from experiments carried out both *in vitro* and *in vivo*. Using cell-free systems, Weitzman and Graceffa (11) showed that a variety of asbestos types can act as catalytic substrates for hydroxyl and superoxide radical generation, apparently from hydrogen peroxide molecules. More recently, Zalma et al. (12) published similar studies on oxygen reduction leading to formation of reactive oxygen radicals on asbestos surfaces. The supposition then is that these radicals will injure pulmonary cells and somehow are involved in fibrogenic and possibly carcinogenic (13) mechanisms.

There is a large list of publications dealing with oxygen metabolism by cells exposed to asbestos fibers. For the purpose of this exercise, it should be sufficient to point out that every cell type tested, i.e., polymorphs (14), macrophages (14,15),

epithelial cells (16), and fibroblasts (17), all produce reactive oxygen species. Upon treatment with asbestos, the mechanisms of radical generation appear to operate through the Haber-Weiss reaction, which results in highly reactive hydroxyl radicals ($\text{OH}\cdot$) and/or through a phosphate oxidase of cell membranes, resulting in a respiratory "burst" and production of reactive oxygen (18). Additional sets of experiments from Holian's laboratory suggest further that asbestos activates oxygen radical production through binding to specific sites on cell membranes (19). Binding of positively charged chrysotile fibers appears to be mediated at least in part by negatively charged terminal sialic acid residues on cell membranes (20,21). Binding, without particle internalization, is sufficient to activate the metabolism of arachidonic acid by lung macrophages (22). Holian suggests that membrane signaling through a "G" protein could activate phospholipase C, which in turn mediates the now classical intracellular signal transduction mechanism through the inositol phosphate pathway, resulting in diacylglycerol and protein kinase C activation and consequent calcium release (23). These events activate membrane-bound oxidases that are capable of reducing molecular oxygen to the bioactive superoxide radical. Studies of asbestos-treated cells using fluphenazine and staurosporine (24) to block protein kinase C support the signal transduction hypothesis. In addition, *in vitro* studies by Kane and her colleagues further define the mechanisms of asbestos-induced cytotoxicity (25). Kane describes this as an oxidant-mediated process associated with lipid peroxidation and other intracellular alterations that lead to irreversible cell death.

The reactive oxygen radicals surely are cytotoxic and are capable of injuring cells. How this cell injury leads to fibrogenic or neoplastic disease remains undefined. Animal models have begun to shed new light on the subject. A most provocative model has been implemented by Mossman at the University of Vermont. Mossman and her colleagues showed that continuous administration of catalase (an antioxidant enzyme) blocked a proportion of the pulmonary inflammatory and fibrogenic responses caused when rats inhaled crocidolite asbestos for 2 weeks (9). This is consistent with other studies from Mossman's laboratory showing that reactive oxygen species play a role in pulmonary cell injury and subsequent proliferation (26). Interestingly, exposures of cells to asbestos *in vitro* and *in vivo* invariably result in the induction of antioxidant enzymes such as superoxide dismutase, glutathione, and catalase as well as ornithine decarboxylase, which appears to play a central role in cell division (13). Studying the regulation of these enzymes at the molecular level has opened new opportunities for understanding the mechanisms of asbestos-induced lung disease.

Growth Factors

The growth factor hypothesis offers an alternative, although not mutually exclusive, set of mechanisms to explain the pathogenesis of asbestos-induced fibrogenesis. It is my view that reactive oxygen species do indeed play a major role in initial cell injury after exposure to fibrogenic particles. However, it also is my view that this injury opens the door for the multitude of cytokines and growth factors that mediate the chemotactic, mitogenic, and fibrogenic phases of interstitial lung disease. The oxygen radicals could be involved in two major events in this regard: stimulation of inflammatory cells to secrete various

factors and injury of the alveolar epithelium, thus allowing movement of factors from the alveoli to the interstitium and/or allowing the proliferation of mesenchymal cells and translocation of interstitial proteins as well as extracellular matrix into alveolar and bronchiolar air spaces. This latter feature is not uncommon in a variety of interstitial fibroproliferative lung diseases (27). In fact, we postulated in 1990 that reactive oxygen molecules were necessary to initially injure the alveolar epithelium, consequently allowing the translocation of platelet-derived growth factor (PDGF) and its high molecular weight binding protein, α_2 -macroglobulin ($\alpha_2\text{M}$) from alveolar spaces to the lung interstitium (28). This hypothesis was tested, in part, by a series of experiments using a co-culture chamber system, wherein a monolayer of primary alveolar epithelial cells was grown on the top of a Nuclepore polycarbonate filter, and early-passage rat lung fibroblasts (RLFs) were cultured on the underside of the same filter (28). When a tight monolayer of epithelium was maintained, 80% of the PDGF with or without $\alpha_2\text{M}$ remained on the epithelial side of the filter for the 3-day period tested. If the monolayer was injured by scraping or more subtly by an oxidant (chlorinated amines generated by mixing taurine with NaOCl), the PDGF and the binding protein readily moved to the fibroblast side of the filter. We do not yet know if other growth factors will behave in a similar manner, and we do not know if this phenomenon takes place *in vivo*. However, it is very clear that chrysotile asbestos exposure causes a "leaky" alveolar epithelium following both months of chronic inhalation (29) or only hours of exposure (30). From these findings, one could assume that serum-derived and inflammatory-cell-derived growth factors were in a suitable location to mediate the pathogenesis of the disease. Just which factors are critical to the process and the mechanisms through which they exert their biological effects are not known, but there are several clues that are considered here.

In approaching the growth factor hypothesis, it is necessary to understand the astounding variety of factors that have been shown to exert multiple biological effects on mesenchymal cells. This concept has been excellently reviewed in several places (31,32). It should become immediately obvious that no one laboratory should (or could) try to study all or even a large segment of these factors. We have selected two factors to try to understand in detail because it appears that they could be playing central roles in the pathogenesis of asbestos-related lung disease and perhaps more generally in interstitial fibro-proliferative disease of various etiologies. The first is PDGF and the second is transforming growth factor β (TGF- β). PDGF is the most potent inducer of mesenchymal cell proliferation known (33), and TGF- β is a powerful stimulator of extracellular matrix proteins by the same cell types (34). Our working hypothesis in the laboratory today is that macrophage and/or fibroblast-derived PDGF is the major mitogen controlling interstitial cell proliferation whereas TGF- β mediates the synthesis of extracellular matrix proteins.

PDGF is an approximately 30,000 D peptide originally purified from human blood platelets (33), and it was shown to be the main reason that serum will support the proliferation of mesenchymal cells in culture. PDGF reportedly acts early in the G_1 phase of the cell cycle, allowing the cells to become "competent" to go through S-phase should they contact a second, or "progression," factor such as insulinlike growth factor (IGF) (35). PDGF exerts its mitogenic effects through interaction with

specific high-affinity membrane receptors found on essentially every mesenchymal cell type tested (36). Normal epithelial and endothelial cells apparently do not possess such receptors. A variety of cell types synthesize and secrete PDGF, including macrophages, fibroblasts, smooth muscle, endothelial, and epithelial cells (33). Human lung macrophages were shown in 1985 to produce PDGF (37), and in 1988 we demonstrated that rat lung macrophages secrete a similar molecule (38). This macrophage product was found to account for the majority of fibroblast proliferation induced by culture medium "conditioned" by macrophages *in vitro* (39). Thus, PDGF, including the macrophage-derived (MD) molecule, is a potent mesenchymal cell mitogen commonly found in cell secretions. But what is the response of macrophages exposed to asbestos fibers and other particles? We have shown that lung macrophages exhibit a concentration and time-dependent increase in the amount of PDGF they secrete after treatment with nonfibrogenic iron spheres or chrysotile asbestos fibers (40). Interestingly, the asbestos induced more PDGF secretion than iron, but differences observed *in vitro* are difficult to interpret because particle sizes, densities, and surface areas are so variable.

We have studied the specific high-affinity receptors for TGF- β and PDGF on the putative target cells, i.e., the rat lung fibroblasts (39,41). These cells, as do all normal mesenchymal cells studied, possess both α - and β -receptor subunits that dimerize in the membrane under the influence of the appropriate ligand, in this case the three isoforms of PDGF known as the AA, AB, and BB dimers (42). The A and B chains are formed from two separate genes. The AA isoform binds only the α - α receptor, the AB isoform binds to both α - α and α - β receptors, and the BB isoforms binds to all three receptor combinations. We showed recently that alveolar and interstitial lung macrophages produce all three isoforms, with BB accounting for about 50% (39). This was important to deduce, because we now know that RLFs exhibit very few α receptors explaining why their chemotactic and mitogenic responses to the AA isoform are very weak (39). Thus, macrophage-derived PDGF is a potent inducer of RLF proliferation because of a predominance of the BB isoform. Most fascinating was our finding that the gene that codes for the α -receptor subunit is upregulated by exposing the fibroblasts to chrysotile asbestos fibers. This altered the behavioral phenotype of the fibroblasts, which responded vigorously to the AA isoform after asbestos treatment. This was due to an upregulation of about 45% of the number of α -receptors on the lung fibroblast membranes (unpublished observations). Whether this phenomenon occurs *in vivo* is not yet known but could be deduced by a combination of immunohistochemistry and *in situ* hybridization techniques. Such studies are ongoing in our laboratory.

The *in vitro* studies described above should be useful in understanding how growth factors work at cell surfaces and whether growth factor production and specific receptor binding can be controlled at the molecular level. But all of this is for naught if there is no evidence of relevant biological events *in vivo*. We have carried out a series of studies on an animal model of asbestosis. These support the contention that growth factors must be operative during various phases of the disease process. In this regard, two compelling sets of morphological data were established (43-45) a) within 24 hr of a brief (1-3 hr) exposure to chrysotile asbestos, epithelial and submucosal cells of the terminal bronchioles, interstitial mesenchymal cells and alveolar

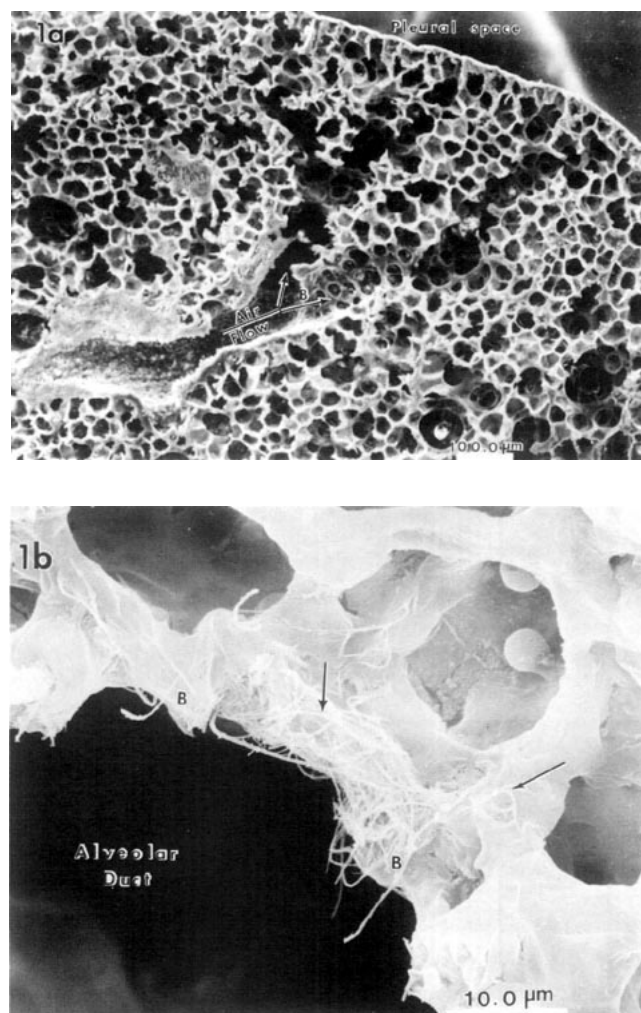


FIGURE 1. (a) Low magnification scanning electron micrograph of the lung parenchyma and a first alveolar duct bifurcation (B) where chrysotile asbestos fibers have been deposited after 1 hr of exposure to approximately 1000 fibers/cc. Note that airflow (arrows) is interrupted by the bifurcation. (b) Higher magnification of a first alveolar duct bifurcation (B) where numerous fibers (arrows) can be readily observed at their sites of original deposition.

epithelial cells of the alveolar ducts, endothelial and smooth muscle cells of small peribronchiolar blood vessels all exhibited highly significant increases in incorporation of tritiated thymidine [^3H]Tdr or bromodeoxyuridine (BrdU). b) A progressive fibrotic lesion developed at the sites where asbestos fibers initially were deposited and lung macrophages accumulated. It is our view that these two features of the asbestos-induced disease are mediated, in part, by PDGF and TGF- β . The following paragraphs describe the development of the lesion.

In 1981 (46), we showed that the majority of inhaled chrysotile asbestos fibers which are small enough to pass through the terminal bronchioles is deposited at the first bifurcation of the alveolar ducts in rats. This was accomplished by exposing the animals for only 0.5 or 1 hr, sacrificing them within 4 min after cessation of exposure, and then counting the fibers at their sites of initial deposition by use of scanning electron microscopy

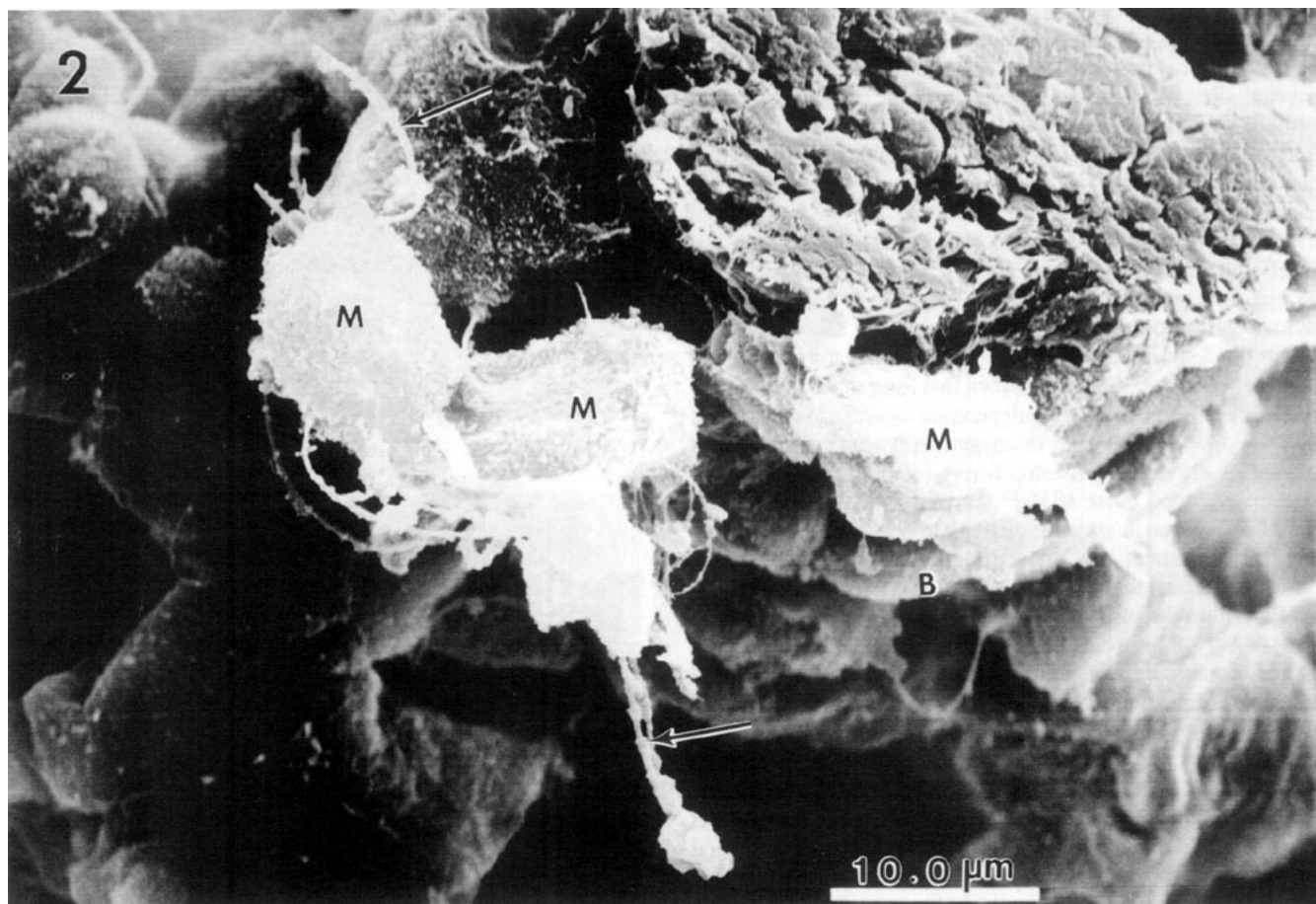


FIGURE 2. Asbestos fibers cause the activation of the fifth component of complement (C5) through the alternative pathway (30,49). The resulting C5a molecules are highly chemotactic for lung macrophages (M) that accumulate at the duct bifurcations during the 12- to 48-hr period after a 1- to 3-hr exposure to chrysotile asbestos (arrows).

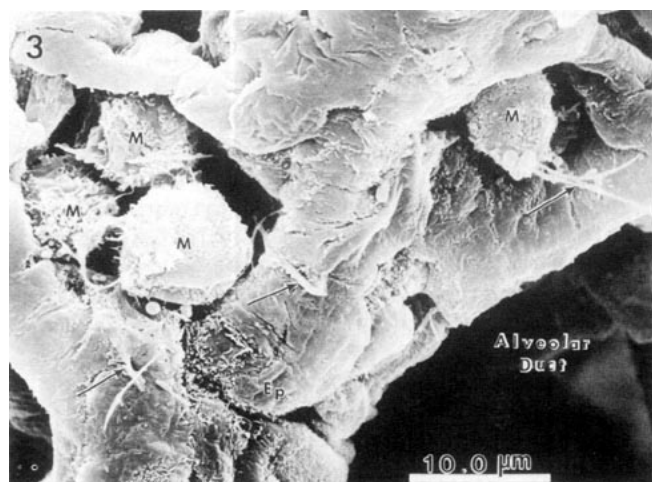


FIGURE 3. The type I epithelium takes up about 20% of the fibers (arrows) that reach the alveolar level, and the fibers are translocated to the underlying interstitium (50,84).

(Fig. 1). In 1983 (47), we demonstrated that this deposition pattern at duct bifurcations was exhibited by a variety of fibers and particles, including crocidolite asbestos, fiberglass, and silica.

A more recent series of studies by Warheit et al. (48) has shown that this initial deposition pattern is characteristic of multiple species and inhaled particles. This deposition pattern is significant because it provides the focal point for the consequent accumulation of both alveolar and interstitial macrophages (45). The alveolar macrophages (AMs) are attracted to the surfaces of the bifurcations by C5a. This powerful chemotactic molecule is generated on the alveolar surfaces by cleavage of the fifth component of complement (C5) through the alternative pathway (30,49). C5 is a normal constituent of the alveolar lining layer and reaches the alveolar surface by protein transudation (30). C5 is consumed, and the C5a concomitantly is elaborated, during the first day following a 1-hr exposure to chrysotile asbestos. Thus, the macrophages begin to arrive at the bifurcations during this time period and reach 10- to 30-fold increases in number by 48-72 hr after exposure (Fig. 2). The alveolar macrophages are essentially gone from the bifurcation surfaces by 1 week after exposure, and normal levels of C5 in the lining layer return by 2 weeks after exposure (30). Although the AMs are cleared, significant numbers of interstitial macrophages remain in the

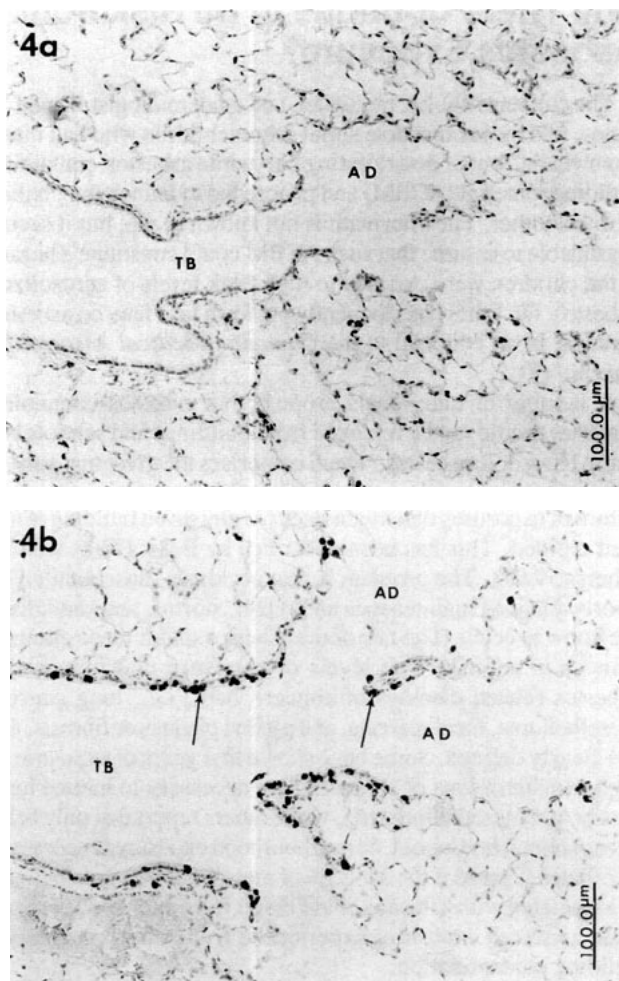


FIGURE 4. Growth factors elaborated by interstitial and alveolar macrophages and probably other cells induce a variety of cell types to proliferate. This is quantified by counting cells (arrows) that have incorporated bromodeoxyuridine during S-phase of the cell cycle. Airway epithelial cells and interstitial cells of the duct bifurcations clearly are labeled in this light micrograph of the bronchiolar-alveolar junction. TB, terminal bronchiole, AD, alveolar duct. (a) Normal, unexposed animal exhibits rare labeled cells; (b) 48 hr after three consecutive 5-hr exposures. (Photo courtesy of P.C. Coin).

connective tissue spaces of the bifurcations (45). This is because numerous asbestos fibers were translocated through the alveolar epithelium to the underlying connective tissue (Fig. 3) (46,50). This fascinating phenomenon began immediately upon deposition of fibers on the bifurcation surfaces (46) and continued for the following 4–5 days until all the fibers had been cleared by the AMs or through the epithelium. In fact, about 20% of the fiber mass that reached the alveolar level was retained in the lung interstitium for at least 6 months after exposure (51). Extrapolating clearance rates supports the view that some percentage of the fibers will be present throughout the animals' lifetime, particularly the long thin fibers that are cleared most slowly (52).

Now one must ask what the consequences are of having asbestos fibers moving through the epithelium, macrophages accumulated on and within duct bifurcations, and fibers in both intra- and extracellular sites in the connective tissue plane. First,

the reader should know that rats and mice exposed to asbestos fibers develop the same lung diseases as humans, i.e., interstitial fibrosis, lung cancer, and mesothelioma (8,53,54). Thus, one might expect that the very earliest events studied in the animal model should tell us something about the pathogenesis of the disease in exposed people.

One of the more striking sequelae of asbestos exposure which we found in rats and mice was the almost 30-fold increase in the percentages of proliferating cells in the terminal bronchioles and alveolar ducts (43,55,56). In normal, unexposed animals and humans, and in rats and mice exposed to high concentrations of nonfibrogenic iron spheres, the turnover rate of epithelial and mesenchymal cells is rather low, something less than 1% at any given point in time (43,56). The turnover rate of endothelial cells of the vasculature is even lower (44). In rats and mice exposed to chrysotile asbestos fibers for 3 hr, the percentages of cells incorporating ^3H -TdR increased dramatically over the 24–72 hr after exposure, as referenced above. Findings were the same using the thymidine analog BrdU in mice exposed for 3 hr and in rats exposed for 3 consecutive days (Fig. 4). The most provocative finding, along with the huge increase in turnover, was the wide range of cell types affected, e.g., bronchiolar epithelial cells and submucosal fibroblasts, endothelial and smooth muscle cells of small pulmonary vessels, alveolar epithelial cells, and interstitial fibroblasts and myofibroblasts. These data compelled us to conclude that a multiplicity of growth factors was diffusing through the lung parenchyma consequent to the asbestos exposure. As one moved away from the first alveolar duct bifurcation, percentages of all the dividing cells were reduced, suggesting that the impetus for cell division emanated from the bifurcation (43,44). This could be the case as the majority of asbestos fibers originally deposited there, and accumulations of macrophages were a consistent feature (Figs. 1 and 2).

Incorporation of thymidine and bromodeoxyuridine was clearly a signal of cell division, not just unscheduled DNA synthesis. This must be the case because several cell populations exhibited increases in volume due to increased cell number as determined by ultrastructural morphometry (44,45,56). First, the alveolar epithelium was shown to increase in thickness as the type II cells divided in response to injury of the type I cells. By 1 month after exposure, the epithelium had returned to normal (45). However, the smooth muscle cells of small vessels at the ends of bronchioles and adjacent to the bifurcations were doubled in number and were twice as thick 1 month after a 3-hr exposure to chrysotile asbestos (44). Furthermore, the interstitial mesenchymal cells of the bifurcations and proximal alveolar duct walls remained significantly elevated in number and volume through the 1-month period studied (45). Within many of the bifurcations were asbestos-containing microcalcifications that were interpreted to be the result of an undefined injury to interstitial cells such as fibroblasts and macrophages, both of which phagocytized fibers within the connective tissue matrix (50), and both of which release increased amounts of PDGF after asbestos treatment (40,57,58; unpublished observations). Finally, there was an increase in extracellular collagen and fibronectin through the 1-month period after exposure (45). This resulted in well-defined scars at the bronchiolar-alveolar duct junctions, primarily at the first and second alveolar duct bifurcations (Fig. 5) (45,46,50,56).

Thus, as little as 1-hr of exposure to chrysotile asbestos causes a fibro-proliferative process, which we believe is the initial

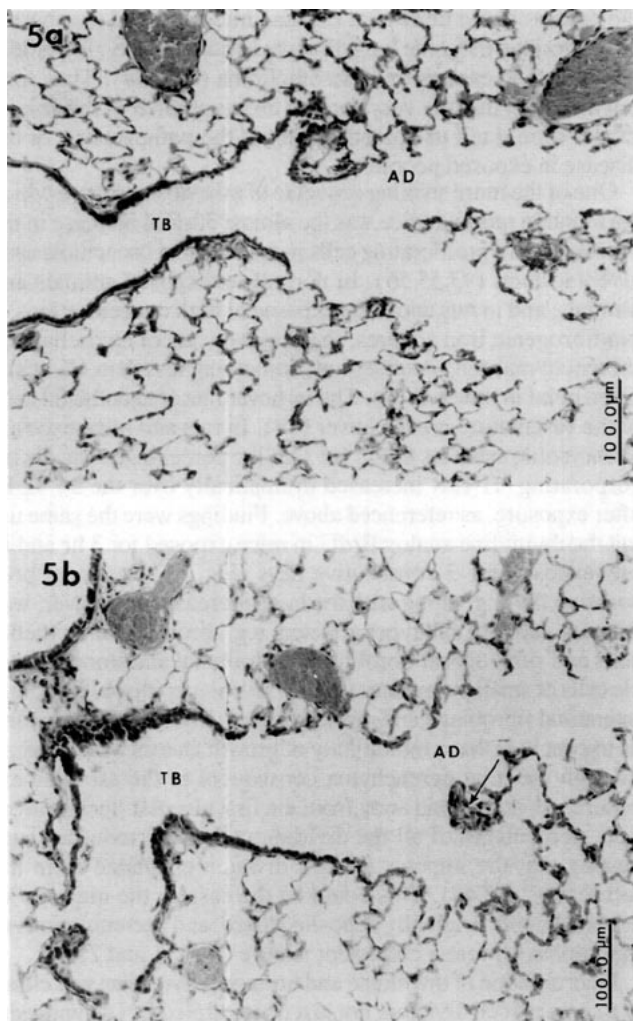


FIGURE 5. Cell proliferation and secretion of collagen and fibronectin consequent to inhalation of chrysotile fibers results in a hypercellular fibrotic lesion (arrow) at the bronchoalveolar duct junctions in the lungs of rats and mice (45,56). These are light micrographs of lung tissue from an unexposed rat (a) and one exposed to chrysotile asbestos for 3 consecutive days and sacrificed 2 weeks after exposure (b) (68). TB, terminal bronchiole; AD, alveolar duct. (Photo courtesy of P. C. Coin).

pulmonary lesion of asbestosis. If the experimental animals or humans exposed to episodic peaks of aerosolized asbestos were to continue to be exposed, this lesion would progress and eventually become diffuse into adjacent regions of the lung parenchyma. What is mediating the cell proliferation and extracellular matrix production? That is the key question, and we do not know the answer. We suggest that growth factors are responsible, possibly stimulated by cytotoxic oxygen radicals. Perhaps TGF- α drives the epithelial response, while PDGF from macrophages is the force behind the fibroblast and smooth muscle cell proliferation. Perhaps fibroblast growth factor from the smooth muscle cells mediates the endothelial cell proliferation, while macrophage-derived TGF- β controls extracellular matrix production. These postulates currently are being tested in our laboratory.

Will There Continue to Be Risk from Asbestos Exposure?

The eminent scholar, physician, and epidemiologist, David V. Bates, told me an anecdote about schoolchildren who had made "snowballs" out of deteriorating chrysotile asbestos-containing building material (ACBM) and proceeded to launch the "balls" at one another. The aftermath is not known to me, but it seems reasonable to assume that such ACBM could constitute a hazard if the children were exposed to high peak levels of aerosolized asbestos. Dr. Bates has eloquently put forth his views on asbestos removal in an editorial to the *Canadian Medical Association Journal* (7).

A number of individuals propose that asbestos-containing material should not be removed from buildings and schools because chrysotile asbestos, which comprises the great majority of fiber in such materials, is a negligible risk factor at low concentrations (6). This issue has not as yet been settled because the nature of exposures that might occur in any given building is not well defined. This has been described by Bates (7) as well as others (59,60). The problem is that accidents, unscheduled or poorly-planned maintenance and repair, storms, and vandalism are known to occur. The fate of the asbestos under these circumstances is unclear. The levels of exposure that induce the asbestos-related diseases of concern here, i.e., lung cancer, mesothelioma, focal scarring, and pleural plaques or fibrosis, are not clearly defined. Some have shown that years of exposure to high concentrations of chrysotile are necessary to induce lung cancer and mesothelioma (6), while others report that only brief occupational, household, or neighborhood exposure is necessary (7). Bates (7) asks if the 20–25 % of mesotheliomas not obviously associated with asbestos could result from such low, perhaps undocumented exposures experienced from environmental or building contamination.

It is becoming increasingly clear that low exposures can induce significant pathobiological responses in humans. Several recent, widely diverse (geographically as well as culturally) populations have exhibited pleural lesions consequent to asbestos exposure. Custodians in the United States (61), railroad-car repair workers in Sweden (62), merchant seamen in Greece (63), and others (64) have developed pleural disease that has compromised their lung function. It is not known if any of these populations are at increased risk of developing mesothelioma. These individuals probably experienced repeated exposures to low concentrations, with brief intense exposures scattered through time. The railroad workers in Sweden were said to have been exposed to between 0.1 and 2.0 fibers/cc (62). As the recent document on asbestos in buildings from the Health Effects Institute clearly points out, the precise nature of the exposures to asbestos in most settings is not well characterized (65). Air monitoring has shown that airborne concentrations in buildings generally are very low (65), but these data tell us nothing about the concentrations of asbestos that could be inhaled during repeated peak episodes if fibers were to be reentrained from surfaces by various activities.

Asbestos is likely to remain a risk for two central reasons: Asbestos, including chrysotile, is a proven carcinogen, and all of human kind's activities cannot be guaranteed to leave asbestos-containing material intact so that significant exposures do not occur. The second point is out of my purview and should be left for

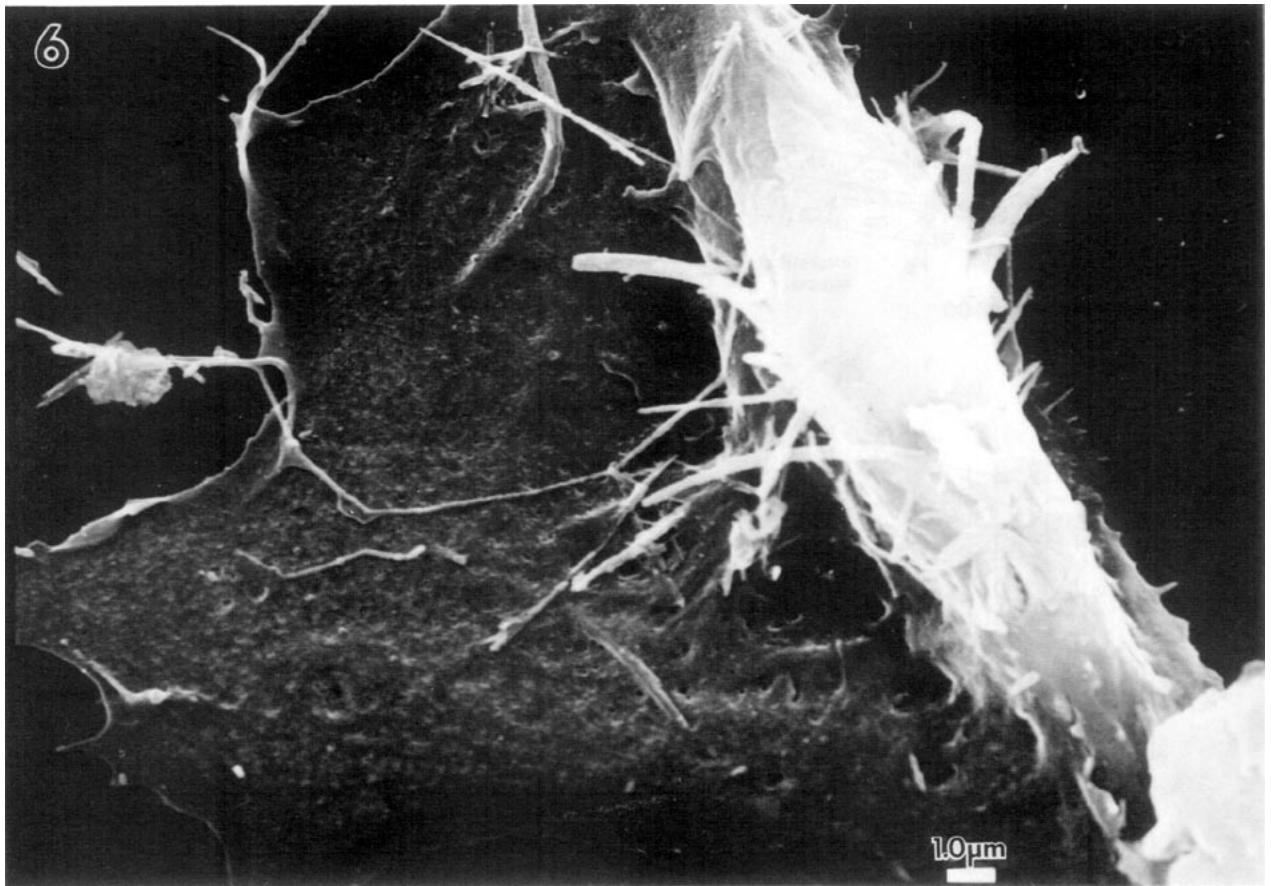


FIGURE 6. Scanning electron micrograph of fibroblast exposed *in vitro* to chrysotile asbestos fibers. After 24 hr in culture, the majority of fibers have accumulated around the nucleus. This distribution allows the fibers to interfere with cell division and normal chromosome segregation (75,78).

engineers and hygienists to sort out. However, the second point is inextricably linked to the first because the biological mechanisms through which asbestos causes cancer are not entirely clear, and the concentrations of fibers that can cause neoplastic transformation are not known. At the molecular and cellular levels, it appears as though relatively few fibers are necessary. A body of data elucidating the potential mechanisms of asbestos-induced carcinogenesis are reviewed below.

Recall that as described above, rats and mice exposed to approximately 1000 fibers/cc of chrysotile asbestos for 1 hr developed a fibroproliferative response that began immediately upon exposure and progressed through at least 1 month after exposure (45,46). When rats were exposed chronically for 1 year to this concentration of chrysotile as a positive control in a study of wollastonite fibers, a significant percentage of the animals developed bronchiolar-alveolar adenocarcinomas and adenomas (66). The very earliest proliferative response (as described above) induced by the first few hours or days of exposure could be critical in the consequent neoplastic process inasmuch as it is becoming increasingly clear that a population of dividing cells is more susceptible to transforming events (67), as will be discussed further. The point here is that a brief, intense exposure to chrysotile asbestos is sufficient to cause dramatic increases in

cell division in experimental animals (43,44,55,56). A second and third day of exposure prolongs the time that the cells proliferate (Coin et al., submitted), and I would expect that subsequent exposures do the same. Further studies are necessary to establish the lowest levels of fiber concentration that are capable of causing a proliferative response and how brief intense (peak) exposures interact with chronic, low dose exposures to induce lung injury. Whether or not an increased rate of cell proliferation is maintained during chronic exposure is an important issue and could be answered by appropriate administration of [^3H]TdR or BrdU to animals during the course of the experiment.

Since we know that chrysotile asbestos causes cell division in animals, and experimental animals develop the same asbestos-related diseases as man, let us assume that asbestos causes increased cell division in humans as well. If so, the transforming events so elegantly described by Barrett (68), Jaurand (69), Hei (70), and others could take place in asbestos-exposed animals and humans. Summarizing these studies briefly, asbestos, including chrysotile, has been described as a nongenotoxic carcinogen because it has not been shown to cause mutations at specific genetic loci in mammalian cells. It is, however, a complete carcinogen, acting as an initiator and tumor promoter (71).

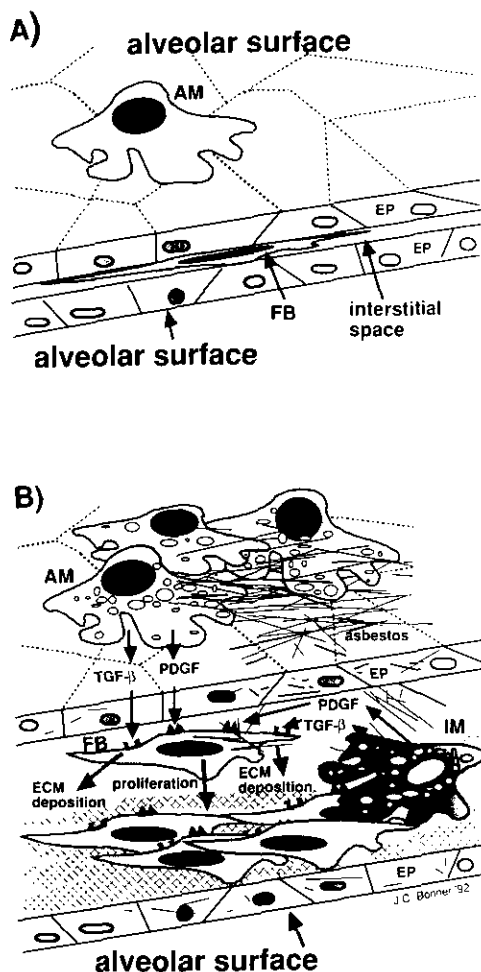


FIGURE 7. Diagram of our hypothetical scheme perhaps explaining in part how macrophage-derived growth factors induce fibroblast growth and connective tissue production through specific receptor-mediated pathways. (A) depicts a normal alveolar wall with an intact epithelium and thin interstitial space. (B) shows that inhaled asbestos fibers attract macrophages, both alveolar (AM) and interstitial (IM) which release growth factors such as platelet-derived growth factor and transforming growth factor β that are capable of stimulating fibroblast growth and secretion of extracellular matrix components. A key event in the disease process is the translocation of fibers from the alveolar surface through the type I epithelium (EP) to the interstitium. The role of oxygen radicals in causing cell injury and the role of growth factors in mediating the fibroproliferative process are currently under intensive study. (Diagram courtesy of J. C. Bonner).

In addition, it is clear that asbestos and other fibers (such as fiberglass) can induce chromosomal mutations in a variety of cell types (72), and this is a good candidate for a mechanism that mediates asbestos carcinogenicity (73). The bulk of this work has been carried out by Barrett and colleagues (74), and recent studies from Cole et al. (75) support this concept and provide new evidence as to how fibers are translocated through epithelial cell cytoplasm to the nucleus by microtubules. The central tenet of the chromosome mutation hypothesis is that intracellular fibers interfere with normal segregation of chromosomes during cell division, thus providing one explanation for the increased

susceptibility of proliferating cells to transforming events. Jaurand showed that chrysotile asbestos alters the growth of mesothelial cells resulting in neoplastic transformation and chromosomal changes (69). The studies of Lechner et al. (76) using human mesothelial cells showed similar results.

In trying to explain how these chromosomal changes might occur, Hesterberg et al. (77), Hesterberg et al. (72), and Oshimura et al. (78,79) exposed embryo fibroblasts to asbestos and fiberglass. They showed that chrysotile asbestos causes highly significantly increases in anaphase abnormalities such as lagging and sticky chromosomes, resulting in aneuploid cells. The process appears to occur because intracellular fibers of varying sizes accumulate around the nucleus within the first few hours of exposure *in vitro* (Fig. 6) (77). Then, as the nuclear envelope dissolves during DNA organization, fibers can interact directly with the metaphase and/or anaphase chromosomes and various components of the spindle apparatus. This leads to abnormal chromosome segregation and cell transformation as discussed above. Whether this mechanism is operative *in vivo* is not known, but it is clear that aneuploidy and other chromosome abnormalities commonly are found in asbestos-induced tumors in both animals and man (80,81). New approaches at the molecular level (82) are likely to elucidate the precise mechanisms through which asbestos and other carcinogenic minerals exert their effects on chromosomes and cause lung tumors.

Conclusions

All varieties of asbestos, including chrysotile, when inhaled distribute along all aspects of the respiratory tract, including the most peripheral portions of the gas-exchanging regions of the lung. Chrysotile, even though many of its fibers are "curly," does indeed reach the periphery of the lung. Numerous chrysotile fibers have been visualized in, and are readily recovered from, the lung parenchymal tissue of humans and experimental animals. A hypothetical scheme of how asbestos might cause a fibroproliferative lesion is depicted in Figure 7.

The initial proliferative events induced by asbestos fibers probably are mediated by a combination of reactive oxygen radicals that injure cells along with growth factors that control cell division. Early cell proliferation could be an integral event in the neoplastic process caused by asbestos.

All varieties of asbestos have been shown to be carcinogens. The precise mechanisms through which they exert tumorigenic effects have not been established. The most consistent experimental evidence supports the view that the fibers interfere with normal chromosome segregation, leading to chromosomal mutations and aneuploidy.

Thousands of tons of asbestos, mostly chrysotile, remain in millions of buildings around the world. Cohen (60) points out that in New York City alone, two-thirds of the 800,000 buildings there contain asbestos, which could be in damaged and deteriorating conditions. It is interesting to note that asbestos-containing building materials have only in the past decade attracted major attention as potential hazards in buildings. In view of the long-term latency period (of 10–40 years) that exists before asbestos-related diseases are manifested clinically, it may not be too soon to become concerned about exposures that could take place if asbestos-containing materials are not properly cared for or disposed of. Cohen (60) and Bates (7) raise a very poignant

issue concerning individuals involved with custodial tasks, maintenance, and repair: they could receive much higher concentrations of fibers than the general population. Cohen further states (60) that even though the expenses associated with asbestos care are huge, "It is legitimate to protect a smaller segment of the population . . . who face large risks. . ."

REFERENCES

- Selikoff, I. J., and Lee, D. H. *Asbestos and Disease*. Academic Press, New York 1978.
- Michaels, L. and Chissick, S. S., Eds. *Asbestos: Properties, Applications, and Hazards*. John Wiley and Sons, New York, 1981.
- Craighead, J. E., Abraham, J. L., Churg, A., Green, F. H., Kleiner, J., Pratt, P. C., Seemayer, T., and Ballyathan, V., and Weill, H. The pathology of asbestos-associated diseases of the lungs and pleural cavities: diagnostic criteria and proposed grading schema. *Arch. Pathol. Lab. Med.* 106: 544-596 (1982).
- Craighead, J. E., and Mossman, B. T. The pathogenesis of asbestos-associated diseases. *N. Engl. J. Med.* 306: 1446-1455. (1982).
- Becklake, M. R. Exposure to asbestos and human disease. *N. Engl. J. Med.* 306: 1480 (1982).
- Mossman, B. T. Bignon, I., Corn, M., Seaton, A., and Gee, J. B. Asbestos: scientific developments and implications for public policy. *Science* 247: 294-301 (1990).
- Bates, D. V. Asbestos: the turbulent interface between science and policy. *Can. Med. Assoc. J.* 144: 554-556 (1991).
- Brody, A. R. An animal model of asbestos-induced interstitial lung disease. In: *CRC Handbook of Animal Models of Pulmonary Disease* (J. O. Cantor, Ed.), CPC Press Inc., Boca Raton, FL, 1989, pp. 183-195.
- Mossman, B. T., Marsh, J. P., Sesko, A., Hill, S., Shatos, M. A., Doherty, J., Petruska, J., Adler, K. B., Hemanway, D., Mickey, R., Vacek, P., and Kagan, E. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene-glycol conjugated catalase in a rapid inhalation model of asbestosis. *Am. Rev. Respir. Dis.* 141: 1266-1271 (1990).
- Harris, C. C., Lechner, J. G., Brinkley, B. R., Eds. *Cellular and Molecular Aspects of Fiber Carcinogenesis*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1991, p. 211.
- Weitzman, S. A., and Graceffa, P. Asbestos catalyzes hydroxyl and superoxide radical release from hydrogen peroxide. *Arch. Biochem. Biophys.* 228: 373-376 (1984).
- Zalma, R., Bonneau, L., Jaurand, M. C., Guignard, J., and Pezerat, H. Formation of oxy-radicals by oxygen reduction arising from the surface activity of asbestos. *Can. J. Chem.* 65: 2338-2345 (1988).
- Mossman, B. T., and Marsh, J. P. Role of active oxygen species in asbestos-induced cytotoxicity cell, proliferation and carcinogenesis. In: *Cellular and Molecular Aspects of Fiber Carcinogenesis* (C. C. Harris, J. G. Lechner, and B. R. Brinkley, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1991 pp. 159-168.
- Donaldson, K., Slight, J., Hannant, D., and Bolton, R. E. Increased release of hydrogen peroxide and superoxide anion from asbestos-primed macrophages. Effect of hydrogen peroxide on the functional activity of alprotease inhibitor. *Inflammation* 9: 133-147 (1985).
- Hansen, K., and Mossman, B. T. Generation of superoxide (O_2^-) from alveolar macrophages exposed to asbestiform and nonfibrous particles. *Cancer Res* 47: 1681-1688 (1987).
- Mossman, B. T., Marsh, J. P., and Shatos, M. A. Alteration of superoxide dismutase activity in tracheal epithelial cells by asbestos and inhibition of cytotoxicity by antioxidants. *Lab. Invest.* 54: 204-212 (1986).
- Meier, B., Radeke, H. H., Selle, S., Younes, M., Sies, H., Resch, K., Habermehl, G. G. Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor- α . *Biochem. J.* 263: 539-543 (1989).
- Mossman, B. T., and Begin, R. O., Eds. *Effects of Mineral Dusts on Cells*. NATO ASI Series, Vol. 30, Springer-Verlag, Heidelberg, 1989, pp. 173-280.
- Roney, P. L., and Holian, A. Possible mechanism of chrysotile asbestos anion-alveolar stimulated superoxide production in guinea pig macrophages. *Tox. Appl. Pharmacol.* 100: 132-144 (1989).
- Brody, A. R., George, G., and Hill, L. H. Interactions of chrysotile and crocidolite asbestos with red blood cell membranes: chrysotile binds to sialic acid. *Lab. Invest.* 49: 468-475 (1983).
- Gallagher, J. E., George, F., and Brody, A. R. Sialic acid mediates the initial binding of positively-charged inorganic particles to alveolar macrophage membranes. *Am. Rev. Respir. Dis.* 135: 1345-1352 (1987).
- Kouzan, S., Gallagher, J., Eling, T., and Brody, A. R. Particle binding to sialic acid residues on macrophage plasma membranes stimulates arachidonic acid metabolism. *Lab. Invest.* 53: 320-327 (1985).
- Holian, A., Roney, P. L. Superoxide anion production by chrysotile asbestos in the guinea pig alveolar macrophage. In: *Effects of Mineral Dusts on Cells*. NATO ASI Series, Vol. 30, Springer-Verlag, Heidelberg, 1989, pp. 223-229.
- Holian, A., Jordan, M. K., Nguyen, H., and Devenyi, Z. J. Inhibition of macrophage activation by isoquinolinesulfonamides, phenothiazines and a naphthalenesulfonamide. *J. Cell Physiol.* 137: 45-54 (1988).
- Goodglick, L. A., Pietras, L. A., and Kane, A. B. Evaluation of the causal relationship between crocidolite asbestos-induced lipid peroxidation and toxicity to macrophages. *Am. Rev. Respir. Dis.* 139: 1265-1273 (1989).
- Sesko, A., Cabot, M., and Mossman, B. T. Hydrolysis of phosphoinositides precedes cellular proliferation in asbestos-stimulated tracheobronchial epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 87: 7385-7390 (1990).
- Antoniades, H. N. Bravo, M. A., Avila, R. E., Galanopoulos, T., Neville-Golden, J., Maxwell, M., and Selman, M. Platelet-derived growth factor on idiopathic pulmonary fibrosis. *J. Clin. Invest.* 86: 1055-1064 (1990).
- Mangum, J. B., Everitt, J. I., Bonner, J. C., Moore, L. R., and Brody, A. R. Coculture of primary pulmonary cells to model alveolar injury and translocation of proteins. *In Vitro Cell Dev. Biol.* 26: 1135-1143 (1990).
- Tetley, T. D., Hext, P. M., Richards, R. J., and McDermott, M. Chrysotile-induced asbestosis: changes in the free cell population, pulmonary surfactant and whole lung tissue in rats. *Br. J. Pathol.* 57: 505-514 (1976).
- Warheit, D. B., Hill, L. H., George, G., and Brody, A. R. Time course of chemotactic factor generation and the corresponding macrophage response to asbestos inhalation. *Am. Rev. Respir. Dis.* 134: 128-133 (1986).
- Nathan, C. Secretory products of macrophages. *J. Clin. Invest.* 79: 319-326 (1987).
- Kelley, J. Cytokines of the lung. *Am. Rev. Respir. Dis.* 141 765-788 (1990).
- Ross, R., Raines, E. W., and Bowen-Pope, D. F. The biology of platelet-derived growth factor. *Cell* 46: 155-169 (1986).
- Pelton, R. W., and Moses, H. L. The beta-type transforming growth factors, mediators of cell regulation in the lung. *Am. Rev. Respir. Dis.* 142: 31-35 (1990).
- Rom, W. N., Basset, P., Fells, G. A., Nukiwa, T., Trapnell, B. C. and Crystal, R. G. Alveolar macrophages release an insulin-like growth factor I-type molecule. *J. Clin. Invest.* 82: 1685-1693 (1988).
- Hart, C. E., Forstrom, J. W., and Kelly, J. D. Two classes of PDGF receptor recognize different isoforms of PDGF. *Science* 240: 1529-1531 (1988).
- Shimokado, K., Raines, E. W., Madtes, D. K., Barrett, T. B., Benditt, E. P., and Ross, R. A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. *Cell* 43: 277-286 (1985).
- Kumar, R. K., Bennett, R. A., and Brody, A. R. A homologue of platelet-derived growth factor produced by rat alveolar macrophages. *FASEB J.* 2: 2272-2277 (1988).
- Bonner, J. C., Osornio-Vargas, A., Badgett, A., and Brody, A. R. Differential proliferation of rat lung fibroblasts induced by the PDGF, AA, AB and BB isoforms secreted by rat alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 5: 539-547 (1991).
- Schapiro, R. M., Osornio-Vargas, A. R., and Brody, A. R. Inorganic particles induce secretion of a macrophage homologue of PDGF in a density and time-dependent manner in vitro. *Exp. Lung Res.* 17: 1011-1024 (1991).
- Kalter, V. G., and Brody, A. R. Receptors for TGF β on rat lung fibroblasts have higher affinity for TGF β 1 than β 2. *Am. J. Respir. Cell Mol. Biol.* 4: 397-407 (1990).
- Seifert, R. A., Hart, C. E., Phillips, J. W., Forstrom, R., Ross, R., Murray, M. J., and Bowen-Pope, D. F. Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. *J. Biol. Chem.* 264: 8771-8778 (1989).
- Brody, A. R., and Overby, L. H. Incorporation of tritiated thymidine by epithelial and interstitial cells in bronchiolar-alveolar regions of asbestos-exposed rats. *Am. J. Pathol.* 134: 133-140 (1990).
- McGavran, P. D., and Brody, A. R. Inhalation of chrysotile asbestos induces rapid cellular proliferation in small pulmonary vessels of mice and rats. *Am. J. Pathol.* 136: 695-705 (1990).
- Chang, L. Y., Overby, L. H., Brody, A. R., and Crapo, J. D. Progressive lung cell reactions and extracellular matrix production after a brief exposure to asbestos. *Am. J. Pathol.* 131: 156-170 (1988).

46. Brody, A. R., Hill, L. H., Adkins, B., Jr., and O'Connor, R. W. Chrysotile asbestos inhalation in rats: deposition pattern and reaction of alveolar epithelium and pulmonary macrophages. *Am. Rev. Respir. Dis.* 128: 670-679 (1981).
47. Brody, A. R., and Roe, M. W. Deposition pattern of inorganic particles at the alveolar level in the lungs of rats and mice. *Am. Rev. Respir. Dis.* 128: 724-729 (1983).
48. Warhelt, D. B., and Hartsky, M. A. Species comparison of proximal alveolar duct deposition patterns of inhaled particles. *Exp. Lung Res.* 16: 81-97 (1990).
49. Warhelt, D. B., George, G., Hill, L. H., Snyderman, R., and Brody, A. R. Inhaled asbestos activates a complement-dependent chemotactic factor for macrophages. *Lab. Invest.* 52: 505-514 (1989).
50. Brody, A. R., and Hill, L. H. Interstitial accumulation of inhaled chrysotile asbestos fibers and consequent formation of microcalcifications. *Am. J. Pathol.* 109: 107-114 (1982).
51. Roggli, V., and Brody, A. R. Changes in numbers and dimensions of chrysotile asbestos fibers in lungs of rats following short-term exposure. *Exp. Lung Res.* 7: 133-147 (1984).
52. Coin, P. C., Roggli, V. L., and Brody, A. R. Deposition, clearance and translocation of chrysotile asbestos from peripheral and central regions of the rat lung. *Environ. Res.* 58: 97-116 (1992).
53. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. The effects of the inhalation of asbestos in rats. *Br. J. Cancer* 29: 252-269 (1974).
54. Bozelka, B. E., Sestini, P., Gaumer, H. R., Hammad, Y., Heather, C. J., and Salvaggio, J. E. A murine model of asbestosis. *Am. J. Pathol.* 112: 326-337 (1983).
55. McGavran, P. D., and Brody, A. R. Chrysotile asbestos inhalation induces tritiated thymidine incorporation by epithelial cells of distal bronchioles. *Am. J. Respir. Cell Mol. Biol.* 1: 231-235 (1989).
56. McGavran, P. D., Butterick, C. J., and Brody, A. R. Tritiated thymidine incorporation and the development of an interstitial lesion in the bronchiolar-alveolar regions of normal and complement deficient mice after inhalation of chrysotile asbestos. *J. Environ. Pathol. Toxicol. Oncol.* 6: 377-392 (1990).
57. Bauman, M. E., Jetten, A. M., Bonner, J. C., Kumar, R. K., Bennett, R. A., and Brody, A. R. Secretion of a platelet-derived growth factor homologue by rat alveolar macrophages exposed to particulates in vitro. *Eur. J. Cell Biol.* 51: 327-334 (1990).
58. Bonner, J. C., and Brody, A. R. Asbestos-induced alveolar injury: evidence for macrophage-derived PDGF as a mediator of the fibrogenic response. *Chest* 99: 545-555 (1991).
59. Aintree-Williams, S., and Preston, J. S. Asbestos and other fiber levels in buildings. *Ann. Occup. Hyg.* 29: 357-366 (1985).
60. Cohen, N. Regulation of in-place asbestos-containing material. *Environ. Res.* 55: 97-106 (1991).
61. Oliver, C. L., Sprince, N. L., and Greene, R. Asbestos-related radiographic abnormalities in public school custodians. *Toxicol. Ind. Health* 6: 629-631 (1990).
62. Hjortsberg, U., Orbaek, P., Arborelius, M., Ranstam, J., and Welinder, H. Railroad workers with pleural plaques: I. Spirometric and nitrogen washout investigation on smoking and non-smoking asbestos-exposed workers. *Am. J. Ind. Med.* 14: 635-641 (1988).
63. Velonakis, W. G., Tzorva, A., Tzonou, A., and Trichopoulos, D. Asbestos-related chest x-ray changes among Greek merchant marine seamen. *Am. J. Ind. Med.* 15: 511-516 (1989).
64. Schwartz, D. A. New developments in asbestos-induced pleural disease. *Chest* 99: 191-198 (1991).
65. HEI. Asbestos in Public and Commercial Buildings. Health Effects Institute, Cambridge, MA, 1991.
66. McConnell, E. E., Hall, L., and Adkins, B. Studies on the chronic toxicity (inhalation) of wollastonite in Fischer 344 rats. *Inhal. Toxicol.* 3: 323-339 (1991).
67. Cohen, S. M., and Ellwein, L. B. Cell proliferation in carcinogenesis. *Science* 249: 1007-1001 (1990).
68. Barrett, J. C., Lamb, P. W., and Wiseman, R. W. Multiple mechanisms for the carcinogenic effects of asbestos and other mineral fibers. *Environ. Health Perspect.* 81: 81-92 (1989).
69. Jaurand, M. C., Kheuang, L., Magne, L., and Bignon J. Chromosomal changes induced by chrysotile fibers or benzo-3,4-pyrene in rat pleural mesothelial cells. *Mutat. Res.* 169: 141 (1986).
70. Hei, T. K., Piao, C. Q., and He, Z. Y. Radiation and asbestos fibers: interaction and mechanisms. 169-182 (1991).
71. Barrett, J. C., Lamb, P. W., and Wiseman, R. W. Hypotheses on the mechanisms of carcinogenesis and cell transformation by asbestos and other mineral dusts. In: *Health Related Effects of Phyllosilicates* (J. Bignon, Ed.), NATO ASI, Vol. G21, 1990, pp. 292-307.
72. Hesterberg, T. W., and Barrett, J. C., Dependence of asbestos and mineral dust-induced transformation of mammalian cells in culture on fiber dimension. *Cancer Res.* 44: 2170-2178 (1984).
73. Hesterberg, T. W., and Barrett, J. C. Induction by asbestos fibers of anaphase abnormalities: mechanism for aneuploidy induction and possibly carcinogenesis. *Carcinogenesis* 6: 473-480 (1985).
74. Hesterberg, T. W., Brody, A. R., Oshimura, M., and Barrett, J. C. Asbestos and silica induce morphological transformation of mammalian cells in culture: a possible mechanism. In: *Silica, Silicosis, and Cancer* (D. F. Goldsmith et al., Eds.), Praeger Press, New York, 1986, pp. 177-185.
75. Cole, R. W., Ault, J. C., Hayden, J. H., and Rieder, C. L. Crocidolite asbestos fibers undergo size-dependent microtubule-mediated transport after endocytosis in vertebrate lung epithelial cells. *Cancer Res.* 51: 4942-4947 (1991).
76. Lechner, J. F., Tokiwa, T., LaVeck, M., Benedict, W. F., Banks-Schlegel, S., Yeager, H., Banerjee, A., Jr., and Harris, C. C. Asbestos-associated chromosomal changes in human mesothelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 82: 3884-3889 (1985).
77. Hesterberg, T. W., Butterick, C. J., Oshimura, M., Brody, A. R., and Barrett, J. C. Role of phagocytosis in Syrian hamster cell transformation and cytogenetic effects induced by asbestos and short and long glass fibers. *Cancer Res.* 46: 5795-5802 (1986).
78. Oshimura, M., Hesterberg, T. W., Tsutsui, T., and Barrett, J. C. Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. *Cancer Res.* 44: 5017-5024 (1984).
79. Oshimura, M., Hesterberg, T. W., and Barrett, J. C. An early nonrandom karyotypic change in immortal Syrian hamster cell lines transformed by asbestos: trisomy of chromosome II. *Cancer Genet. Cytogenet.* 22: 225-231 (1986).
80. Gibas, Z., Li, F. P., Antman, K. H., Bernai, S., Stahel, R., and Sandberg, A. A. Chromosome changes in malignant mesothelioma. *Cancer Genet. Cytogenet.* 20: 191-197 (1986).
81. Stenman, C., Olofsson, K., Masson, B., Hagman, and Mark, J. Chromosomes and chromosomal evolution in human mesotheliomas as reflected in sequential analyses of two cases. *Hereditas* 105: 233-238 (1986).
82. Barrett, J. C., and Wiseman, R. Cellular and molecular mechanisms of multistep carcinogenesis: relevance of carcinogen risk assessment. *Environ. Health Perspect.* 76: 75-87 (1987).
83. Pinkerton, K. E., Brody, A. R., Miller, F. J., and Crapo, J. D. Exposure to low levels of ozone results in enhanced pulmonary retention of inhaled asbestos fibers. *Am. Rev. Respir. Dis.* 140: 1075-1081 (1989).